REMARKS/ARGUMENTS

Claims 1-3, 5-21, 23-58 and 60-65 are active in this application. Support for the definition of the glycoprotein as a lysosomal hydrolase is found, for example, in Claim 4 (now cancelled) and the specification as originally filed. No new matter is added. Favorable reconsideration is requested.

The rejection of Claims 1 and 56 under 35 U.S.C. § 102(e) over Mulligan et al (U.S. patent no. 6,224,858) is respectfully traversed.

Mulligan et al do not describe producing a lysosomal hydrolase with reduced complex carbohydrates as claimed. Accordingly, withdrawal of this ground of rejection is requested.

The rejection of Claims 2-6, 19-24, and 57-61 under 35 U.S.C § 103(a) over the combination of Van Hove et al, Mulligan et al, and Gottlieb is respectfully traversed.

Applicant states on page 2, lines 13-15: "Lectin resistant cell lines, in general, are known (Stanley (1983) Meth. Enzymology 96:157-189; Gottlieb et al (1974) Proc. Nat. Acad. Sci., U.S.A., 71(4):1078-1082; Stanley et al (1990) Somat Cell Mol Genet (3):211-223)."

In addition on pages 3-4, the Applicant describes again that lectin resistant cell lines were known and therefore to produce a lysosomal hydrolase with reduced complex carbohydrates, one could use a known lectin resistant cell line and introduce a expression vector carrying the polynucleotide that would encode the lysosomal hydrolase.

This is, in fact, what is commonly done in the field as demonstrated by the attached listing of Abstracts obtained from the PubMed database. For example:

(1) Parcyk (FEBS Lett. 1991 278(2):267-70) describes the expression of a viral glycoprotein in lectin resistant Madin-Darby canine kidney (MDCK) cells.

(2) Hughes and Mills (J Cell Physiol 1996, 128(3):402-12) describes the expression of various glycoproteins in lectin resistant Baby hamster kidney) BHK cells.

(3) Yu et al (Mol Genet Metab. 2000, 71(4):573-80) describes the production of the lysosomal enzyme alpha-N-acetylglucosaminidase (involved in Sanfilippo syndrome type B) in lectin resistant Chinese Hamster Ovary (CHO) cells.

(4) Carbonneau and Stanners (Cell Adhes Commun. 1999, 7(3):233-44) describes the production of human carcinoembryonic antigen (CEA) in lectin resistant CHO cells.

(5) Nagayama et al (J Biol Chem. 1998, 273(50) :33423-8) describes the production of tyrotropin receptor in lectin resistant cells.

(6) Fuller et al (Biochim Biophys Acta, 1998, 406(3):283-90) describes the production of the lysosomal enzyme N-acetylgalactosamine-4-sulphatase (involved in Mucopolysaccharidosis type IV) in lectin resistant CHO cells.

(7) Fenouillet et al (Virology, 1996, 218(1):224-31) describes the production of HIV membrane glycoproteins in lectin resistant CHO cells.

(8) Grossman et al (J Biol Chem, 1995, 270(49):29378-85) describes the expression of thyrotropin in lectin resistant CHO cells.

(9) Haspel et al (J Cell Physiol, 1988, 136(2):361-6) describes the expression of hexose transporter glycoprotein in lectin resistant CHO cells.

(10) Koyama and Hughes (J Biol Chem, 1992, 267(36):25939-44) describes the expression of integrins in lectin resistant BHK cells.

As further discussed on page 4, lines 2-9:

> However, in attempts to transform a lectin resistant cell line in order to express a non-native glycoprotein, e.g., acid α-glucosidase, the amount of protein expressed and thus recovered was very poor thereby having little practical utility.
>
> The present inventors have discovered quite unexpectedly that when a mammalian cell is transfected to express a glycoprotein of interest is subjected to lectin selection, one is able to obtain both high levels of glycoprotein expression coupled with a reduction in complex carbohydrates on the glycoproteins' surface are observed.

The present claims provide introducing the polynucleotide encoding the lysosomal hydrolase and then culturing the transfected cell with a lectin to obtain a lectin resistant cell which expresses the lysosomal hydrolase. Therefore, the order in which the steps of the claimed method are performed were found to be important for producing a lysosomal hydrolase with reduced complex carbohydrates practicable. The lack of success in producing a lysosomal hydrolase by transfecting a lectin resistant cell is described in the application on page 4, which is also reproduced above. The success of producing lysosomal hydrolases according to the claimed method is demonstrated in the Examples section of the present application, see pages 23-24.

As the cited prior art provides no description or suggestion that the order in which the introducing and selecting must be performed when producing a lysosomal hydrolase with reduced complex carbohydrates, the claims would not have been obvious. Furthermore, the fact that transfecting a lectin resistant cell did not work relative to the claimed method demonstrates that the present claims would not have been obvious. Therefore, withdrawal of this ground of rejection is requested.

The rejection of Claims 7, 11-14, 16, 17, 25, 29, 30-32, 34, 35 and 62-64 under 35 U.S.C § 103(a) over the combination of Van Hove et al, Mulligan et al, and Gottlieb further in view of Bao et al and Kornfeld et al is respectfully traversed.

This ground of rejection is to various dependent claims which add the additional steps of contacting with a GlcNAc-phosphotransferase and/or a phosphodiester α-GlcNAcase. In particular, the Bao et al and Kornfeld et al are relied upon by the Examiner to provide disclosure for these claims. However, for the reasons discussed above, the combination of prior art does not provide any description or suggestion that the order in which the introducing and selecting is performed is important for producing lysosomal hydrolases in mammalian cells. Furthermore, the fact that transfecting a lectin resistant cell did not work relative to the claimed method demonstrates that the present claims would not have been obvious. As a result, Claims 7, 11-14, 16, 17, 25, 29, 30-32, 24, 25 and 62-64 would not have been obvious in view of the cited prior art.

Withdrawal of this ground of rejection is requested.

The rejection of Claims 11, 12, 16, 29, 30 and 34 under 35 U.S.C. § 112, second paragraph is respectfully traversed.

The essential inquiry pertaining to the requirement under 35 U.S.C. § 112, second paragraph is whether the claims set out and circumscribe a particular subject matter with a reasonable degree of clarity and particularity. Definiteness of claim language must be analyzed, not in a vacuum, but in light of:

(A) The content of the particular application disclosure;

(B) The teachings of the prior art; and

(C) The claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. See MPEP § 2173.02

The phrase "stringent conditions" defines a class of polynucleotides having at least 70% identity to the recited polynucleotide sequence (see page 20 of the application).

The determination of "stringent conditions" is well within the knowledge in the field.

Identity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA--DNA hybrids, the T_m can be approximated from the equation of *Meinkoth and Wahl*, Anal. Biochem., 138:267-284 (1984): T_m =81.5°C.+16.6 (log M)+0.41 (%GC)-0.61 (% form)-500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C. for each 1% of mismatching; thus, T_m, hybridization and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with approximately 90% identity are sought, the T_m can be decreased 10°C.

Therefore, based on the definition of polynucleotides that hybridize under stringent conditions (page 20) combined with the common knowledge in the relevant field, the claims are definite.

Withdrawal of this rejection is requested.

Applicant requests that the rejection of Claims 1-17, 19-35 and 56-64 under the doctrine of obviousness-type double patenting over copending application no. 10/023,889 be held in abeyance as the claims in 10/023,889 have not yet been patented.

Applicant previously filed two Information Disclosure Statements, one on September 13, 2003 and one on July 23, 2003. However, return signed copies of those Statements have not been returned to the Applicant. Accordingly, Applicant requests a signed and dated copy

of those Information Disclosure Statements as evidence of the Examiner's consideration of the publications listed therein.

Additionally, Applicant is filing herewith another Information Disclosure Statement of a International Examination report and cited references from a related international PCT application. Applicant requests a return signed and dated copy of this IDS as well.

Finally, Applicant requests allowance of this application.

Respectfully submitted,

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1: Eur J Biochem. 1987 Oct 15;168(2):287-94. Related Articles, Links

Characterization of a mannosidase acting on alpha 1----3- and alpha 1----6-linked mannose residues of oligomannosidic intermediates of glycoprotein processing.

Monis E, Bonay P, Hughes RC.

National Institute for Medical Research, London, England.

Baby hamster kidney (BHK) cell extracts catalyze the conversion of [3H]mannose-labelled (Man)5GlcNAc and (Man)6GlcNAc oligosaccharides to a (Man)3GlcNAc species that retains affinity for concanavalin-A-Sepharose and appears to be Man alpha 1----3[Man alpha 1----6]Man beta 1----4GlcNAc. The properties of the (Man)5GlcNAc-hydrolase activity differ from lysosomal alpha-mannosidases as well as previously described processing mannosidases acting on oligosaccharide intermediates of N-glycan assembly. Mosquito cell extracts catalyze hydrolysis of (Man)6GlcNAc but lack the (Man)5GlcNAc hydrolase activity detected in BHK cell extracts. Glycopeptide analysis has been carried out on a ricin-resistant BHK mutant RicR14 that lacks N-acetylglucosaminyl transferase I and fails to convert oligomannosidic N-glycans to complex-type chains, and mosquito cells that constitutively lack N-acetylglucosaminyl transferase I. In both cell lines, the cellular glycoproteins contain (Man)5GlcNAc oligosaccharide as the major stable component equivalent to a 15-20-fold increase compared with normal BHK cells. Although containing very high amounts of asparagine-linked (Man)5(GlcNAc)2, RicR14 cells exhibit (Man)5GlcNAc hydrolase activity at levels similar to wild-type BHK cells. This result, together with previous work [Foddy, L., Feeney, J. & Hughes, R.C. (1986) Biochem. J. 233, 697-706] showing the complete inhibition of conversion of oligomannosidic intermediates to complex-type N-glycans in BHK cells treated with swainsonine, an inhibitor of mannosidase II but not the (Man)5GlcNAc hydrolase activity, argues against a major role for the (Man)5GlcNAc hydrolase activity in N-glycan assembly and suggesting other functions for the mannosidase activity in BHK cells.

PMID: 3665925 [PubMed - indexed for MEDLINE]

: Eur J Biochem. 1986 Jul 15;158(2):227-37. Related Articles, Links

Ricin-resistant mutants of baby-hamster-kidney cells deficient in alpha-mannosidase-II-catalyzed processing of asparagine-linked oligosaccharides.

Hughes RC, Feeney J.

Previous work has shown that two ricin-resistant mutants of baby hamster kidney (BHK) cells, RicR15 and RicR19, synthesize only hybrid and oligomannose-type asparagine-linked oligosaccharides [Hughes, R. C. and Mills, G. (1985) Biochem. J. 226, 487-498]. In the present report glycopeptides were released from disrupted cells by exhaustive digestion with pronase, fractionated by chromatography on concanavalin-A--Sepharose, DEAE-Sephacel and lentil-lectin--Sepharose and characterized by 500-MHz 1H-NMR spectroscopy. The major hybrid structure identified in both cell lines contains five mannose residues and the sequence NeuNAc alpha 2----3Gal beta 1----4GlcNAc beta 1----2 linked to the alpha 1----3 arm mannose of the core pentasaccharide. Analysis of extracts of normal or mutant cells has shown in the mutants a deficiency in alpha-mannosidase activity measured with p-nitrophenyl alpha-mannoside. This activity is swainsonine-sensitive and exhibits a pH optimum at about 6-6.5. Assays using a specific substrate for alpha-mannosidase II, a terminal processing glycosidase in conversion of penta-mannose hybrid intermediates to complex N-glycans, reveals a reduced activity in RicR15 cells. Analysis of glycopeptides obtained from cells labelled with [3H]fucose or [3H]galactose revealed a small proportion of branched complex N-glycans of normal structure in mutant cells.

PMID: 3732270 [PubMed - indexed for MEDLINE]

2: FEBS Lett. 1991 Jan 28;278(2):267-70. Related Articles, Links

The role of carbohydrates in vectorial exocytosis. The secretion of the gp 80 glycoprotein complex in a ricin-resistant mutant of MDCK cells.

Parczyk K, Koch-Brandt C.

Institut fur Biochemie, Abteilung Molekulare Genetik, Universitat Frankfurt, Germany.

In the polarized epithelial Madin-Darby canine kidney (MDCK) cell line an 80 kDa glycoprotein complex (gp 80) is sorted into the apical pathway of exocytosis and is secreted constitutively at the apical cell surface. The unglycosylated form of the protein complex is secreted in a nonpolar fashion at both surface domains [(1987) J. Cell. Biol. 105, 2735-2743]. Using ricin-resistant MDCK cells the role of the terminal galactose and sialic acid residues in the sorting of the gp 80 complex was analysed. The results suggest that the carbohydrate cores, rather than the ultimate or penultimate sugar residues, play a critical role in the intracellular transport of this protein.

PMID: 1899391 [PubMed - indexed for MEDLINE]

3: J Biol Chem. 1990 Dec 5;265(34):21269-78. Related Articles, Links

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Endocytic membrane traffic to the Golgi apparatus in a regulated secretory cell line.

Green SA, Kelly RB.

Department of Biochemistry and Biophysics, University of California, San Francisco 94143-0448.

We have established a ricin-resistant glycosylation-defective PC12 pheochromocytoma cell line to study biochemically glycoprotein traffic from the cell surface to the Golgi apparatus in regulated secretory cells. The strategy employed in this study is a modification of that used previously (Duncan, J. R., and Kornfeld, S. (1988) J. Cell Biol. 106, 617-628) to demonstrate transport of the cation-independent and -dependent mannose 6-phosphate receptors from the cell surface to the trans-Golgi network in nonsecretory cell types. In ricin-resistant PC12 cells, radiolabeled galactose was incorporated enzymatically into surface glycoconjugates, primarily glycoproteins. Resistance to beta-galactosidase was acquired upon reculture at 37 degrees C due to further terminal glycosylation of the galactose residues. Treatment of N-linked oligosaccharides isolated from recultured cells with a variety of glycosidases in conjunction with beta-galactosidase demonstrated the addition of sialic acid N-acetylglucosamine and fucose residues to the galactose residues in recultured cells. Resistance to beta-galactosidase was not acquired in cells recultured at 19 degrees C, indicating that subsequent glycosylation of galactose residues did not occur at the cell surface or in endosomes. While glycosylation of galactose incorporated into asparagine oligosaccharides in Chinese hamster ovary clone 13 cells was not significant (less than 1%) after 6 h of reculture, approximately 10% of the galactose incorporated into surface oligosaccharides was further glycosylated in PC12 cells in this time. Analysis of total labeled versus beta-galactosidase-resistant proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis demonstrated that endocytic traffic to the site of glycosylation activity in mutant PC12 cells was highly selective, but included a much greater number of proteins than were detected in Chinese hamster ovary clone 13 fibroblasts.

PMID: 2123489 [PubMed - indexed for MEDLINE]

4: J Biol Chem. 1988 Apr 5;263(10):4895-9. Related Articles, Links

Lectin-like binding of pertussis toxin to a 165-kilodalton Chinese hamster ovary cell glycoprotein.

Brennan MJ, David JL, Kenimer JG, Manclark CR.

Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892.

Chinese hamster ovary (CHO) cells cluster in the presence of pertussis toxin, a response that is correlated with the ADP-ribosylation of a Mr = 41,000 membrane protein by the toxin. A ricin-resistant line of CHO cells (CHO-15B) which specifically lacks the terminal NeuAc----Gal beta 4GlcNAc oligosaccharide sequence on glycoproteins did not cluster in response to pertussis toxin. These cells do contain the Mr = 41,000 protein substrate for the enzymatic activity of the toxin which suggests that pertussis toxin, like certain plant lectins, does not bind to or is not internalized by the CHO-15B cells. There was no evidence of pertussis toxin binding to gangliosides or neutral glycolipids isolated from CHO cells but the toxin bound to a Mr = 165,000 component in N-octyglucoside extracts of CHO cells that had been separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electroblotted to nitrocellulose. Plant lectins from Ricinus communis and Erythina cristagalli detected a similar size band in CHO cells and also did not react with CHO-15B cells. Unlike pertussis toxin, these plant lectins recognized two other major bands in CHO cell extracts and reacted best after sialidase treatment of nitrocellulose transfers containing CHO cell extracts. Conversely, sialidase treatment abolished binding a pertussis toxin and wheat germ agglutinin, a plant lectin that reacts with multivalent sialic acid residues on glycoproteins, to the Mr = 165,000 band. Purified B oligomer of pertussis toxin also uniquely detected a Mr = 165,000 component in CHO cell extracts while the A subunit of pertussis toxin was unreactive. These results indicate that pertussis toxin binds to a CHO cell glycoprotein with N-linked oligosaccharides and that sialic acid contributes to the complementary receptor site for the toxin. In addition, they suggest that a glycoprotein may serve as a cell surface receptor for pertussis toxin and that this interaction is mediated by a lectin-like binding site located on the B oligomer.

PMID: 3350815 [PubMed - indexed for MEDLINE]

5: J Cell Physiol. 1986 Sep;128(3):402-12. Related Articles, Links

Functional differences in the interactions of glycosylation-deficient cell lines with fibronectin, laminin, and type IV collagen.

Hughes RC, Mills G.

Fibronectin isolated from the conditioned medium of monolayer cultures of baby hamster kidney (BHK) cells and several ricin-resistant (Ric) mutants derived from them express differences in N-glycosylation. The asparagine-linked oligosaccharides of BHK cell-derived fibronectin consist largely of complex chains, whereas hybrid and/or high-mannose chains are present in the fibronectins of mutant cell lines. The fibronectins exhibiting different glycosylation patterns are incorporated to similar extents into the cell-layer of human skin fibroblasts. In contrast, mutant cells retain significantly less endogenously produced fibronectin than BHK cells and also incorporate less human cellular fibronectin into a pericellular matrix. In vitro adhesion assays show that mutant cells attach to and spread relatively poorly on fibronectin-or type IV collagen-coated substrata but interact as well as do BHK cells with a laminin substratum. These results indicate that asparagine-linked oligosaccharides of fibronectin are not required for the binding and incorporation of the molecule into cell layers, but, as constituents of other cellular glycoproteins, they do modulate the ability of BHK cells to interact with some matrix components.

PMID: 2943748 [PubMed - indexed for MEDLINE]

6: Biochem J. 1986 Feb 1;233(3):697-706. Related Articles, Links

Properties of baby-hamster kidney (BHK) cells treated with Swainsonine, an inhibitor of glycoprotein processing. Comparison with ricin-resistant BHK-cell mutants.

Foddy L, Feeney J, Hughes RC.

Baby-hamster kidney (BHK) cells were grown continuously in long-term monolayer culture in the presence of Swainsonine, an inhibitor of alpha-mannosidase II, a processing enzyme involved in glycoprotein biosynthesis. The asparagine-linked oligosaccharides (N-glycans) were isolated from Pronase-digested cells by gel filtration, ion-exchange chromatography and affinity chromatography on concanavalin A--Sepharose and lentil lectin--Sepharose. The major N-glycans, analysed by 500 MHz 1H-n.m.r. spectroscopy, were identified as hybrid structures containing five mannose residues and neutral high-mannose N-glycans. The major hybrid species contained a core-substituted fucose alpha(1----6) residue and a NeuNAc alpha(2----3)Gal beta(1----4)GlcNAc terminal sequence; smaller amounts of non-sialylated and non-fucosylated hybrid structures were also detected. Swainsonine-treated cells also produced neutral oligosaccharides containing a single reducing N-acetylglucosamine residue substituted with polymannose sequences. The glycopeptide composition of Swainsonine-treated BHK cells resembles closely that of the ricin-resistant BHK cell mutant, RicR21 [P. A. Gleeson, J. Feeney and R. C. Hughes (1985)

Biochemistry 24, 493-503], except the hybrid structures of RicR21 cells contain three, not five, mannose residues. Like RicR21 cells, Swainsonine-treated BHK cells showed a greatly increased resistance to ricin cytotoxicity, but not to modeccin, another galactose-binding lectin. These effects were readily reversed on removal of Swainsonine and growth in normal medium.

PMID: 3085652 [PubMed - indexed for MEDLINE]

7: Biochemistry. 1985 Jan 15;24(2):493-503. Related Articles, Links

Structures of N-glycans of a ricin-resistant mutant of baby hamster kidney cells. Synthesis of high-mannose and hybrid N-glycans.

Gleeson PA, Feeney J, Hughes RC.

The asparagine-linked glycopeptides (N-glycans) of a ricin-resistant mutant of baby hamster kidney (BHK) cells, RicR21, have been isolated and fractionated from a Pronase digest of disrupted cells by concanavalin A (Con A)-Sepharose chromatography, ion-exchange chromatography, and lentil lectin chromatography. The structures of all the major N-glycans have been determined by 500-MHz H NMR spectroscopy. RicR21 synthesizes only hybrid and high-mannose N-glycans. All the hybrid structures contain only three mannose residues. The major hybrid glycopeptide has the following structure: (Formula: see text). There is also about 15% of the nonfucosylated species present. Only a small amount (less than or equal to 5%) of the asialo hybrid is produced. Branched hybrid N-glycans are also present in RicR21 cells, containing two complex antenna linked beta 1----2 and beta 1----4 to the Man alpha 1----3 arm; about 70% of this species is core fucosylated. Man6GlcNAc2 glycopeptide is the most abundant (about 70%) of the high-mannose N-glycans. These studies account for the very poor ricin binding property of this mutant, as the sialic acid residues of the major hybrid N-glycan are exclusively linked alpha 2----3 to galactose and ricin is unable to bind to alpha 2----3-substituted galactosyl residues [Baenziger, J. U., & Fiete, D. (1979) J. Biol. Chem. 254, 9795-9799].

PMID: 3978088 [PubMed - indexed for MEDLINE]

8: J Virol. 1983 Oct;48(1):325-9. Related Articles, Links

N-acetylgalactosaminyltransferase activity involved in O-glycosylation of herpes simplex virus type 1 glycoproteins.

Serafini-Cessi F, Dall'Olio F, Scannavini M, Costanzo F, Campadelli-Fiume

G.

We report on N-acetylgalactosaminyltransferase (UDPacetylgalactosamine--protein acetylgalactosaminyltransferase; EC 2.4.1.41) activity in herpes simplex virus type 1 (HSV-1)-infected BHK and RicR14 cells, a line of ricin-resistant BHK cells defective in N-acetylglucosaminyltransferase I. The enzyme catalyzed the transfer of [14C]N-acetylgalactosamine (GalNAc) from UDP-[14C]GalNAc into HSV glycoproteins, as identified by immunoprecipitation. The sugar was selectively incorporated into the immature forms of herpesvirus glycoproteins pgC, pgD, and gA-pgB, which are known to contain N-linked glycans of the high-mannose type. The high incorporation of [14C]GalNAc into endogenous acceptors of HSV-1-infected RicR14 cells was consistent with the accumulation of immature forms of HSV glycoproteins which occurs in these cells. Mild alkaline borohydride treatment of glycoproteins labeled via GalNAc transferase showed that the transferred GalNAc was O-linked and represented the first sugar added to the peptide backbone.

PMID: 6310156 [PubMed - indexed for MEDLINE]

9: Carbohydr Res. 1983 Aug 16;120:215-34. Related Articles, Links

Hybrid, sialylated N-glycans accumulate in a ricin-resistant mutant of baby hamster kidney BHK cells.

Hughes RC, Mills G, Stojanovic D.

Glycoproteins synthesized in a ricin-resistant mutant of BHK cells, clone RICR21, were labelled by growth of the cells in radioactive D-mannose, D-glucosamine, or L-fucose. Glycopeptides obtained from disrupted cells by exhaustive digestion with Pronase were fractionated into components binding to concanavalin A-Sepharose and nonbinding components. The binding components eluted with methyl alpha-D-mannopyranoside were separated by gel filtration on Bio-Gel P-4 into two main subfractions: an oligomannosidic fraction that was susceptible to Jack bean alpha-D-mannosidase and a fraction that became totally degraded only in the additional presence of neuraminidase, beta-D-galactosidase, and N-acetyl-beta-D-glucosaminidase. Further analysis of the latter fraction by exoglycosidase digestion together with consideration of the known pathways for the biosynthesis of asparagine-linked sugar chains of glycoproteins was consistent with a "hybrid" structure containing a NeuAc leads to Gal leads to GlcNAc sequence linked to the alpha-D-mannosyl-(1 leads to 3) residue of the core sequence, and a terminal alpha-D-mannosyl group linked to the alpha-(1 leads to 6) branch of the core sequence. The hybrid fraction was labelled after growth of the cells in radioactive L-fucose and was adsorbed to a lentil lectin-Sepharose column indicating the presence of core fucosylation. The novel structure represented about 30-35% of the total cellular glycopeptides of RICR21 cells and was not present in the

glycopeptides of normal, ricin-sensitive BHK cells. Conversely, double-branched (biantennary) complex N-glycans, a prominent constituent of BHK cell glycoproteins, were absent in RICR21 cells, and analysis of the nonbinding fraction obtained from concanavalin A-Sepharose indicated that triple- and quadruple-branched (tri- and tetra-antennary), complex N-glycans present in normal BHK cell glycoproteins were also absent.

PMID: 6627248 [PubMed - indexed for MEDLINE]

10: Biochem J. 1983 Jun 1;211(3):575-87. Related Articles, Links

Analysis by lectin affinity chromatography of N-linked glycans of BHK cells and ricin-resistant mutants.

Hughes RC, Mills G.

Normal baby hamster kidney (BHK) fibroblasts and ricin-resistant (RicR) mutants of BHK cells derived from them were labelled metabolically with [3H]mannose or [3H]fucose. Glycopeptides obtained by digestion of disrupted cells with Pronase were separated by affinity chromatography on concanavalin A-Sepharose. In the normal BHK cells major glycopeptide fractions were obtained consisting of tetra- and tri-antennary sialylated complex glycans, bi-antennary sialylated glycans, and neutral oligomannosidic chains. The majority of bi-antennary chains were shown to contain a fucosyl-(alpha 1-6)-N-acetylglucosaminyl sequence in the core region by their ability to bind to a lentil lectin affinity column. All of the mutant cell lines examined were found to accumulate oligomannosidic glycans in cellular glycoproteins: complex sialylated glycans were either absent or greatly reduced in amount. Analysis of fractions isolated from concanavalin A-Sepharose by Bio-Gel P-4 chromatography and glycosidase degradation indicated that the glycans accumulating in RicR14 cells have the general structure: (formula; see text) and derivatives having fewer alpha-mannosyl units. We have also analysed the glycopeptides released by trypsin treatment from the surface of the normal and mutant cells, as well as those obtained by proteolysis of fibronectin isolated from the medium. The glycopeptide profiles of the cell-surface-derived material and of fibronectin showed for the mutant cells a marked accumulation of oligomannosidic chains at the expense of complex oligosaccharide chains. Hence, the alterations in glycan structure detected in bulk cellular glycoproteins of RicR cells are expressed also in cell surface glycoproteins and in fibronectin, a secreted glycoprotein.

PMID: 6882361 [PubMed - indexed for MEDLINE]

11: J Virol. 1982 Sep;43(3):1061-71. Related Articles, Links

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Infectivity and glycoprotein processing of herpes simplex virus type 1 grown in a ricin-resistant cell line deficient in N-acetylglucosaminyl transferase I.

Campadelli-Fiume G, Poletti L, Dall'Olio F, Serafini-Cessi F.

We report on the replication of herpes simplex virus type 1 (HSV-1) and viral glycoprotein processing in RicR14 cells, a mutant ricin-resistant cell line defective in N-acetylglucosaminyl transferase I activity. In these cells HSV-1(MP) and (F) replicated to yields very similar to those in parental BHK cells. The kinetics of HSV-1 adsorption in mutant and in parent cells was also essentially identical. Progeny virions from ricin-resistant and wild-type cells displayed comparable specific infectivities. However, in the mutant cells the efficiency of plating of progeny virus from both RicR14 and BHK cells was reduced. HSV-1(MP) failed to induce syncytia in RicR14 cells either in a plaque assay or after a high-multiplicity infection. Moreover, the fully glycosylated forms of glycoproteins (gB, gC, and gD) were totally absent, and only the partially glycosylated precursors (pgC, pgD. and a triplet in the gB-gA region) accumulated in HSV-1-infected ricin-resistant cells and in herpesvirions made in these cells. Consistent with these results analysis of pronase glycopeptides from cells labeled with [14C]glucosamine showed a strong decrease of sialylated complex-type oligosaccharides and a dramatic accumulation of the neutral mannose-rich chains. The latter chains predominate in partially glycosylated precursors, whereas the complex acidic chains predominate in the fully processed forms of HSV glycoproteins. These results taken together indicate that (i) host-cell N-acetylglucosaminyl transferase I participates in the processing of HSV glycoproteins; and (ii) infectivity of herpesvirions does not necessarily require the mature form of gB. The absence of HSV-1(MP)-induced fusion in RicR14 cells is discussed.

PMID: 6292449 [PubMed - indexed for MEDLINE]

12: FEBS Lett. 1982 May 3;141(1):14-8. Related Articles, Links

Interaction of ricin-sensitive and ricin-resistant cell lines with other carbohydrate-binding toxins.

Sargiacomo M, Hughes RC.

PMID: 7084475 [PubMed - indexed for MEDLINE]

13: Biochim Biophys Acta. 1979 Jan 5;550(1):100-9. Related Articles, Links

Two-dimensional electrophoresis of surface glycoproteins of normal BHK cells and ricin resistant mutants.

Pena SD, Mills G, Hughes RC.

The surface glycoproteins of baby hamster kidney (BHK) cells were iodinated by lactoperoxidase and submitted to a two-dimensional electrophoresis procedure involving isoelectric focusing in the first dimension and SDS gel electrophoresis in the second dimension. After autoradiography a complex but reproducible pattern was obtained. The technique was then applied to the study of three ricin-resistant mutant clones with reduced rates of cell-cell and/or cell-substratum adhesion. Abnormal patterns were observed in all three mutant clones indicating different mechanisms of ricin resistance and identifying glycoproteins which may be involved in cellular interactions.

PMID: 760785 [PubMed - indexed for MEDLINE]

14: Mol Genet Metab. 2000 Dec;71(4):573-80. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Short-term enzyme replacement in the murine model of Sanfilippo syndrome type B.

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The Sanfilippo syndrome type B (MPS III B) is an autosomal recessive disease caused by deficiency of alpha-N-acetylglucosaminidase (EC 3. 2.1.50), one of the lysosomal enzymes required for the degradation of heparan sulfate. The disease is characterized by profound neurodegeneration but relatively mild somatic manifestations, and is usually fatal in the second decade. A mouse model had been generated by disruption of the Naglu gene in order to facilitate the study of pathogenesis and the development of therapy for this currently untreatable disease. Recombinant human alpha-N-acetylglucosaminidase (rhNAGLU) was prepared from secretions of Lec1 mutant Chinese hamster ovary cells. The enzyme, which has only unphosphorylated high-mannose carbohydrate chains, was endocytosed by mouse peritoneal macrophages via mannose receptors, with half-maximal uptake at ca. 10(-7) M. When administered intravenously to 3 month-old mice, rhNAGLU was taken up avidly by liver and spleen but marginally if at all by

thymus, lung, kidney, heart, and brain (in order of diminishing uptake). The half-life of the enzyme was 2.5 days in liver and spleen. Immunohistochemistry and electron microscopy showed that only macrophages were involved in enzyme uptake and correction in these two organs, yet the storage of glycosaminoglycan was reduced to almost normal levels. The results show that the macrophage-targeted rhNAGLU can substantially reduce the body burden of glycosaminoglycan storage in the mouse model of Sanfilippo syndrome III B. Copyright 2000 Academic Press.

PMID: 11136549 [PubMed - indexed for MEDLINE]

15: Protein Expr Purif. 2000 Jun;19(1):202-11. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Purification and characterization of recombinant human alpha-N-acetylglucosaminidase secreted by Chinese hamster ovary cells.

Zhao KW, Neufeld EF.

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alpha-N-Acetylglucosaminidase (EC 3.2.1.50) is a lysosomal enzyme that is deficient in the genetic disorder Sanfilippo syndrome type B. To study the human enzyme, we expressed its cDNA in Lec1 mutant Chinese hamster ovary (CHO) cells, which do not synthesize complex oligosaccharides. The enzyme was purified to apparent homogeneity from culture medium by chromatography on concanavalin A-Sepharose, Poros 20-heparin, and aminooctyl-agarose. The purified enzyme migrated as a single band of 83 kDa on SDS-PAGE and as two peaks corresponding to monomeric and dimeric forms on Sephacryl-300. It had an apparent K(m) of 0.22 mM toward 4-methylumbelliferyl-alpha-N-acetylglucosaminide and was competitively inhibited by two potential transition analogs, 2-acetamido-1,2-dideoxynojirimycin (K(i) = 0.45 microM) and 6-acetamido-6-deoxycastanospermine (K(i) = 0.087 microM). Activity was also inhibited by mercurials but not by N-ethylmaleimide or iodoacetamide, suggesting the presence of essential sulfhydryl residues that are buried. The purified enzyme preparation corrected the abnormal [(35)S]glycosaminoglycan catabolism of Sanfilippo B fibroblasts in a mannose 6-phosphate-inhibitable manner, but its effectiveness was surprisingly low. Metabolic labeling experiments showed that the recombinant alpha-N-acetylglucosaminidase secreted by CHO cells had only a trace of mannose 6-phosphate, probably derived from contaminating endogenous CHO enzyme. This contrasts with the presence of mannose 6-phosphate on naturally occurring alpha-N-acetylglucosaminidase secreted by diploid human fibroblasts and on recombinant human alpha-l-

iduronidase secreted by the same CHO cells. Thus contrary to current belief, overexpressing CHO cells do not necessarily secrete recombinant lysosomal enzyme with the mannose 6-phosphate-targeting signal; this finding has implications for the preparation of such enzymes for therapeutic purposes.

PMID: 10833408 [PubMed - indexed for MEDLINE]

16: Cell Adhes Commun. 1999;7(3):233-44. Related Articles, Links

Role of carbohydrate structures in CEA-mediated intercellular adhesion.

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Human carcinoembryonic antigen (CEA) is a member of a family of cell surface glycoproteins representing a subset of the immunoglobulin superfamily and is a major tumor marker. CEA has been demonstrated to function in vitro, at least, as a homotypic intercellular adhesion molecule. CEA can also inhibit the differentiation of several different cell types and contribute to tumorigenesis, an activity that requires CEA-CEA interactions. Post-translational modifications that could modulate CEA-CEA binding are therefore of interest. CEA is heavily glycosylated with 28 consensus sites for the addition of asparagine-linked carbohydrate structures, leading to a molecule with a bottle brush-like structure. In order to modulate the glycosylation of CEA, we transfected the functional cDNA of CEA into Chinese hamster ovary (CHO) mutant cells, Lec1, Lec2, and Lec8, which are deficient in enzymes responsible for various steps in the glycosylation processing pathway. Aggregation assays of cells in suspension were performed with stable CEA transfectants of these cell lines and showed that all of the aberrant CEA glycoforms could still mediate adhesion. In addition, the specificity of adhesion of these glycoforms was unchanged, as shown by homotypic and heterotypic adhesion assays between the transfectants. Lec1 and Lec2 transfectants did, however, show an increased speed and final extent of aggregation, which is consistent with models in which sugar structures interfere with binding through protein domains. Lec8 transfectants, on the other hand, with more truncated sugar structures than Lec2, showed less aggregation than wild type (WT) transfectants. We therefore conclude that carbohydrates do not determine the adhesion property of CEA or its specificity, in spite of the unusually high degree of glycosylation; they do, however, modulate the strength of adhesion.

PMID: 10626907 [PubMed - indexed for MEDLINE]

17: Glycobiology. 1998 Dec;8(12):1173-82. Related Articles, Links

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Recycling cell surface glycoproteins undergo limited oligosaccharide reprocessing in LEC1 mutant Chinese hamster ovary cells.

Reichner JS, Helgemo SL, Hart GW.

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The ability of particular cell surface glycoproteins to recycle and become exposed to individual Golgi enzymes has been demonstrated. This study was designed to determine whether endocytic trafficking includes significant reentry into the overall oligosaccharide processing pathway. The Lec1 mutant of Chinese hamster ovary (CHO) cells lack N -acetylglucosaminyltransferase I (GlcNAc-TI) activity resulting in surface expression of incompletely processed Man5GlcNAc2 N -linked oligosaccharides. An oligosaccharide tracer was created by exoglycosylation of cell surface glycoproteins with purified porcine GlcNAc-TI and UDP-[3H]GlcNAc. Upon reculturing, all cell surface glycoproteins that acquired [3H]GlcNAc were acted upon by intracellular mannosidase II, the next enzyme in the Golgi processing pathway of complex N -linked oligosaccharides (t1/2= 3-4 h). That all radiolabeled cell surface glycoproteins were included in this endocytic pathway indicates a common intracellular compartment into which endocytosed cell surface glycoproteins return. Significantly, no evidence was found for continued oligosaccharide processing consistent with transit through the latter cisternae of the Golgi apparatus. These data indicate that, although recycling plasma membrane glycoproteins can be reexposed to individual Golgi-derived enzymes, significant reentry into the overall contiguous processing pathway is not evident.

PMID: 9858639 [PubMed - indexed for MEDLINE]

18: J Biol Chem. 1998 Dec 11;273(50):33423-8. Related Articles, Links

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Role of asparagine-linked oligosaccharides in protein folding, membrane targeting, and thyrotropin and autoantibody binding of the human thyrotropin receptor.

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The amino-terminal ectodomain of thyrotropin (TSH) receptor (TSHR) is heavily glycosylated with asparagine-linked (N-linked) oligosaccharides. The present studies were designed to evaluate how acquisition and processing of N-linked oligosaccharides play a role in the functional maturation of human TSHR. A glycosylation inhibitor tunicamycin, which inhibits the first step of N-linked glycosylation (acquisition of N-linked oligosaccharides), and a series of mutant Chinese hamster ovary (CHO)-Lec cells defective in the different steps of glycosylation processing were used. Inhibition of acquisition of N-linked oligosaccharides by tunicamycin treatment in CHO cells stably expressing TSHR produced nonglycosylated TSHR, which was totally nonfunctional. In contrast, all of the TSHRs synthesized in mutant CHO-Lec1, 2, and 8 cells (mannose-rich, sialic acid-deficient, and galactose-deficient oligosaccharides, respectively) bound TSH and produced cAMP in response to TSH with an affinity and an EC50 similar to those in TSHR expressed in parental CHO cells (CHO-TSHR; sialylated oligosaccharides). However, Lec1-TSHR and Lec2-TSHR were not efficiently expressed on the cell surface, whereas the expression levels of Lec8-TSHR and CHO-TSHR were essentially identical. All of the TSHRs expressed in CHO-Lec cells cleaved into two subunits. Finally, anti-TSHR autoantibodies from Graves' patients interacted with all of the TSHRs harboring different oligosaccharides to a similar extent. These data demonstrate that acquisition and processing of N-linked oligosaccharides of TSHR appear to be essential for correct folding in the endoplasmic reticulum and for cell surface targeting in the Golgi apparatus. We also show that complex type carbohydrates are not crucially involved in the interaction of TSHR with TSH and anti-TSHR autoantibodies.

PMID: 9837919 [PubMed - indexed for MEDLINE]

19: Biochim Biophys Acta. 1998 Apr 28;1406(3):283-90. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Receptor mediated binding of two glycosylation forms of N-acetylgalactosamine-4-sulphatase.

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The lysosomal storage disorders are a group of inherited metabolic diseases each characterised by a relative or absolute deficiency of one or more of the lysosomal proteins involved in the hydrolysis of glycoconjugates or in the transport of the resulting product. Enzyme replacement therapies are under consideration for a number of these disorders and are based on the in vitro observation that cells from affected patients can be corrected by addition of exogenous enzyme. In this study, two glycosylation variants of the lysosomal enzyme N-acetylgalactosamine-4-sulphatase (4S) (the deficiency of which causes Mucopolysaccharidosis (MPS) type VI, (Maroteaux-Lamy syndrome) were made by expression of 4S cDNA in both wild type chinese hamster ovary (CHO-K1), and Lec1 (N-acetylglucosaminyltransferase I deficient CHO-K1) cells. Differences in the glycosylation pattern of the two enzyme forms were demonstrated with endoglycosidase H and N-glycosidase F digestions. The receptor mediated binding of these two forms of 4S to two cell types, human skin fibroblasts and rat alveolar macrophages, was then analysed. We have shown that both enzyme forms bind to the mannose-6-phosphate receptor on human skin fibroblasts with equal affinity demonstrating that the degree of phosphorylation of mannose residues in the two forms is similar. However, using rat alveolar macrophages, we found that the binding/uptake of the two enzymes differs considerably. These results show that differences in glycosylation of lysosomal enzymes can be an important factor in altering enzyme uptake by different cell types. Thus, producing carbohydrate modification variants in this way may be useful for altering the distribution of exogenous enzyme in vivo.

PMID: 9630676 [PubMed - indexed for MEDLINE]

20: Virology. 1996 Apr 1;218(1):224-31. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Biological properties of recombinant HIV envelope synthesized in CHO glycosylation-mutant cell lines.

Fenouillet E, Miquelis R, Drillien R.

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N-glycosylation of the human immunodeficiency virus type-1 envelope (Env) glycoprotein precursor (gp160) occurs by transfer of Glc3Man9GlcNAc2 onto the nascent protein. Maturation then occurs via cleavage of the three Glc residues, which starts during translation. These events are considered necessary to create Env functional conformation: treatment with "alpha"-glucosidase inhibitors, but not alpha-mannosidase inhibitors (i) impairs gp160 cleavage into gp120 and gp41,

(ii) diminishes the accessibility of gp120 V3 region, (iii) prevents gp120 binding to its CD4 receptor, and (iv) prevents gp41-mediated membrane fusion. These inhibitors are of therapeutic interest. Here, using a collection of parent and mutant CHO cells that possess mutations in different steps of glycosylation, we reassessed the role of glycans in both the processing and the properties of recombinant gp160 expressed from a vaccinia virus vector. Mutant cells were as follows: Lec23 (which lacks alpha-glucosidase I activity) produces a collection of triglucosylated structures (Glc3Man7-9GlcNAc2); LEC10 (which has increased GlcNAc transferase III activity) produces complex glycans with a bisected GlcNAc residue; Lec1 (which lacks GlcNAc transferase I) and Lec3.2.8.1 (which lacks GlcNAc transferase I and has decreased activity of CMP-NeuNAc and UDP-Gal translocases) produce Man5GlcNAc2 glycans at complex or hybrid sites. As expected, glycosylation of Env produced from mutants was affected but, irrespective of the glycosylation phenotype, (i) similar quantities of Env were synthesized, (ii) the immunoreactivity of V3 was similar, (iii) gp160 was efficiently cleaved into gp120 and gp41, (vi) Env was exposed at the cell membrane, (v) secreted gp120 bound CD4, and (vi) membrane gp41 was able to induce membrane fusion with CD4+ cells. Thus, the glycosylation alterations examined are dispensable for Env processing and biological activity in CHO cells. In particular, removal of the three outer Glc residues was not required per se for Env folding in this system because functional Env is obtained from Lec23 cells: it appears therefore that lack of modification is not equivalent to drug inhibition of modification. These data are discussed in the light of previous reports describing the use of glycosidase inhibitors to alter glycosylation.

PMID: 8615025 [PubMed - indexed for MEDLINE]

21: J Biol Chem. 1995 Dec 8;270(49):29378-85. Related Articles, Links

Expression of human thyrotropin in cell lines with different glycosylation patterns combined with mutagenesis of specific glycosylation sites. Characterization of a novel role for the oligosaccharides in the in vitro and in vivo bioactivity.

Grossmann M, Szkudlinski MW, Tropea JE, Bishop LA, Thotakura NR, Schofield PR, Weintraub BD.

Molecular and Cellular Endocrinology Branch, NIDDK, National Institutes of Health, Bethesda, Maryland 20892-1758, USA.

We used a novel approach to study the role of the Asn-linked oligosaccharides for human thyrotropin (hTSH) activity. Mutagenesis of Asn (N) within individual

glycosylation recognition sequences to Gln (Q) was combined with expression of wild type and mutant hTSH in cell lines with different glycosylation patterns. The in vitro activity of hTSH lacking the Asn alpha 52 oligosaccharide (alpha Q52/TSH beta) expressed in CHO-K1 cells (sialylated oligosaccharides) was increased 6-fold compared with wild type, whereas the activities of alpha Q78/TSH beta and alpha/TSH beta Q23 were increased 2-3-fold. Deletion of the Asn alpha 52 oligosaccharide also increased the thyrotropic activity of human chorionic gonadotropin, in contrast to previous findings at its native receptor. The in vitro activity of wild type hTSH expressed in CHO-LEC2 cells (sialic acid-deficient oligosaccharides), CHO-LEC1 cells (Man5GlcNAc2 intermediates), and 293 cells (sulfated oligosaccharides) was 5-8-fold higher than of wild type from CHO-K1 cells. In contrast to CHO-K1 cells, there was no difference in the activity between wild type and selectively deglycosylated mutants expressed in these cell lines. Thus, in hTSH, the oligosaccharide at Asn alpha 52 and, specifically, its terminal sialic acid residues attenuate in vitro activity, in contrast to the previously reported stimulatory role of this chain for human chorionic gonadotropin and human follitropin activity. The increased thyrotropic activity of alpha Q52/CG beta suggests that receptor-related mechanisms may be responsible for these differences among the glycoprotein hormones. Despite their increased in vitro activity, alpha Q52/TSH beta, and alpha Q78/TSH beta from CHO-K1 cells had a faster serum disappearance rate and decreased effect on T4 production in mice. These findings highlight the importance of individual oligosaccharides in maintaining circulatory half-life and hence in vivo activity of hTSH.

PMID: 7493973 [PubMed - indexed for MEDLINE]

22: Glycobiology. 1994 Dec;4(6):895-901. Related Articles, Links

Core fucosylation of high-mannose-type oligosaccharides in GlcNAc transferase I-deficient (Lec1) CHO cells.

Lin AI, Philipsberg GA, Haltiwanger RS.

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During studies on the fucosylation of endogenous proteins in parental (Pro5) and N-acetyl-D-glucosamine (GlcNAc) transferase I-deficient (Lec1) Chinese hamster ovary (CHO) cells, we observed that Lec1 cells incorporate approximately 10-fold less [3H]fucose into macromolecules than Pro5 cells. Interestingly, most of the labelled oligosaccharides from both cell types could be released from the macromolecules by digestion with peptide N-glycosidase F (PNGase F). This was unexpected for Lec1 cells because they do not synthesize complex- or hybrid-type N-glycans. Structural analyses of the fucosylated oligosaccharides from Lec1 cells showed the fucose to be in an alpha 1,6 linkage to the core GlcNAc of

relatively small oligomannose N-glycans (Man4GlcNAc2 and Man5GlcNAc2, where Man is D-mannose). Comparing the sizes of oligomannose N-glycans from Pro5 and Lec1 cells demonstrated a much higher proportion of the small (Man4GlcNAc2 and Man5GlcNAc2) oligomannose species in Lec1 cells. These results suggest that the core alpha 1,6 fucosyltransferase will fucosylate small (Man4-Man5GlcNAc2), but not large (Man8-Man9GlcNAc2) oligomannose N-glycans.

PMID: 7734851 [PubMed - indexed for MEDLINE]

23: Glycobiology. 1993 Dec;3(6):589-96. Related Articles, Links

O-linked fucose in glycoproteins from Chinese hamster ovary cells.

Stults NL, Cummings RD.

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We report our discovery that many glycoproteins synthesized by Chinese hamster ovary (CHO) cells contain fucose in O-glycosidic linkage to polypeptide. To enrich for the possible presence of O-linked fucose, we studied the lectin-resistant mutant of CHO cells known as Lec1. Lec1 cells lack N-acetylglucosaminyltransferase I and are therefore unable to synthesize complex-type N-linked oligosaccharides. Lec1 cells were metabolically radiolabelled with [6-3H]fucose and total glycoproteins were isolated. Glycopeptides were prepared by proteolysis and fractionated by chromatography on a column of concanavalin A (Con A)-Sepharose. The sets of fractionated glycopeptides were treated with mild base/borohydride to effect the beta-elimination reaction and release potential O-linked fucosyl residues. The beta-elimination produced [3H]fucitol quantitatively from [3H]fucose-labelled glycopeptides not bound by Con A-Sepharose, whereas none was generated by treatment of glycopeptides bound by the lectin. The total [3H]fucose-labelled glycoproteins from Lec1 cells were separated by SDS-PAGE and detected by fluorography. Treatment of selected bands of detectable glycoproteins with mild base/borohydride quantitatively generated [3H]fucitol. Pretreatment of the glycoproteins with N-glycanase prior to the SDS-PAGE method of analysis caused an enrichment in the percentage of radioactivity recovered as [3H]fucitol. Trypsin treatment of [3H]fucose-labelled intact CHO cells released glycopeptides that contained O-linked fucose, indicating that it is present in surface glycoproteins. These findings demonstrate that many glycoproteins from CHO cells contain O-linked fucosyl residues and raise new questions about its biosynthesis and possible function.

PMID: 8130391 [PubMed - indexed for MEDLINE]

24: J Cell Physiol. 1988 Aug;136(2):361-6. Related Articles, Links

Structure, biosynthesis, and function of the hexose transporter in Chinese hamster ovary cells deficient in N-acetylglucosaminyltransferase 1 activity.

Haspel HC, Revillame J, Rosen OM.

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We have used a Chinese hamster ovary cell line deficient in N-acetylglucosaminyltransferase 1 activity (Lec1) to study the effects of altered asparagine-linked oligosaccharides on the structure, biosynthesis, and function of glucose transporter protein. Immunoblots of membranes of Lec1 cells show a glucose transporter protein of Mr 40,000, whereas membranes of wild-type (WT) cells contain a broadly migrating Mr 55,000 form similar to that observed in several other mammalian tissues. The total content of immunoreactive glucose transporters in Lec1 cells is 3.5-fold greater than that of WT cells. Digestion with endoglycosidases, treatment with inhibitors of glycosylation, and interactions with agarose-bound lectins demonstrate that glucose transporters of both cell lines derive from a similar Mr 38,000 core polypeptide and that both contain asparagine-linked oligosaccharide. Transporters in Lec1 cells contain primarily "undecorated" but "trimmed" mannose-type asparagine-linked oligosaccharides, while the protein in WT cells contains a mixture of "decorated" and "trimmed" asparagine-linked oligosaccharides. Biosynthetic and turnover studies demonstrate that Lec1 cells, in contrast to WT cells, are unable fully to process the core asparagine-linked oligosaccharides of maturing glucose transporters. When radiolabeled in methionine-deficient medium both Lec1 and WT cells show similar rates of synthesis and turnover of glucose transporter proteins. It should be noted, however, that starvation for a critical amino acid may alter the ability of the cell to synthesize or degrade proteins. The abilities of Lec1 and WT cells to transport hexoses and to interact with the inhibitor cytochalasin B are very similar. The results indicate that, although altered asparagine-linked glycosylation can affect the content and biogenesis of glucose transporters, these changes do not greatly modify cellular hexose uptake. The possibility that alterations in asparagine-linked glycosylation may change the cell surface localization or acquisition of a "functional conformation" of the glucose transporter is also suggested.

PMID: 2970467 [PubMed - indexed for MEDLINE]

25: J Biol Chem. 1987 Jun 5;262(16):7575-9. Related Articles, Links

The role of asparagine-linked carbohydrate in natural killer cell-mediated cytolysis.

Ahrens PB, Ankel H.

Chinese hamster ovary cell lines with specific lesions in the formation of glycoconjugates were tested for their sensitivity to lysis by interferon-boosted human natural killer cells. We report here that the type of asparagine-linked carbohydrate present on target cell glycoproteins determines their susceptibility to natural killer lysis. The targets tested were Chinese hamster ovary parent cells and Lec1, Lec2, and Lec8 mutants. Lec8 and Lec2 cells show an overall reduction of galactose and/or sialic acid in their glycoconjugates due to defects in the translocation of UDP-galactose and CMP-sialic acid, respectively. Due to a specific block in N-linked carbohydrate processing, Lec1 cells produce only high mannose-type oligosaccharides, but their glycolipids are identical to those of the parent. Both Lec2 and Lec8 mutants are more sensitive to natural killer lysis than the parent cells. This is consistent with their extensive reduction in cell surface sialic acid. Furthermore, Lec1 mutants are more susceptible to natural killer lysis than the parent cells. To confirm that the increased natural killer sensitivity of Lec1 cells was due to the modification of N-linked carbohydrate, parent cells were treated with swainsonine, a specific inhibitor of N-linked oligosaccharide processing. Swainsonine-treated parent cells are nearly as sensitive to natural killer lysis as the Lec1 mutants.

PMID: 3108253 [PubMed - indexed for MEDLINE]

26: Mol Cell Biol. 1985 Jun;5(6):1204-11. Related Articles, Links

Control of carbohydrate processing: the lec1A CHO mutation results in partial loss of N-acetylglucosaminyltransferase I activity.

Stanley P, Chaney W.

Lec1 CHO cell glycosylation mutants are defective in N-acetylglucosaminyltransferase I (GlcNAc-TI) activity and therefore cannot convert the oligomannosyl intermediate (Man5GlcNAc2Asn) into complex carbohydrates. Lec1A CHO cell mutants have been shown to belong to the same genetic complementation group but exhibit different phenotypic properties. Evidence is presented that lec1A represents a new mutation at the lec1 locus resulting in partial loss of GlcNAc-TI activity. Structural studies of the carbohydrates associated with vesicular stomatitis virus grown in Lec1A cells (Lec1A/VSV) revealed the presence of biantennary and branched complex

carbohydrates as well as the processing intermediate Man5GlcNAc2Asn. By contrast, the glycopeptides from virus grown in CHO cells (CHO/VSV) possessed only fully processed complex carbohydrates, whereas those from Lec1/VSV were almost solely of the Man5GlcNAc2Asn intermediate type. Therefore, the Lec1A glycosylation phenotype appears to result from the partial processing of N-linked carbohydrates because of reduced GlcNAc-TI action on membrane glycoproteins. Genetic experiments provided evidence that lec1A is a single mutation affecting GlcNAc-TI activity. Lec1A mutants could be isolated at frequencies of 10(-5) to 10(-6) from unmutagenized CHO cell populations by single-step selection, a rate inconsistent with two mutations. In addition, segregants selected from Lec1A X parental cell hybrid populations expressed only Lec1A or related lectin-resistant phenotypes and did not include any with a Lec1 phenotype. The Lec1A mutant should be of interest for studies on the mechanisms that control carbohydrate processing in animal cells and the effects of reduced GlcNAc-TI activity on the glycosylation, translocation, and compartmentalization of cellular glycoproteins.

PMID: 2993857 [PubMed - indexed for MEDLINE]

27: Parasitology. 1994 May;108 (Pt 4):397-405. Related Articles, Links

Ricin-resistant mutants of Leishmania major which express modified lipophosphoglycan remain infective for mice.

Cappai R, Morris L, Aebischer T, Bacic A, Curtis JM, Kelleher M, McLeod KS, Moody SF, Osborn AH, Handman E.

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Glycosylation variants of the virulent Leishmania major clone V121 were generated by mutagenesis with N-methyl-N-nitroso-N-nitroguanidine and selected using the galactose-specific lectin Ricinus communis II (RCA II). Three mutants, 4B9, 1D1 and 1C12, which failed to bind RCA II, were found to have an altered expression of lipophosphoglycan (LPG), a molecule implicated in the attachment to host macrophages and survival within the phagolysosome. There were differences in the antigenicity, molecular weight and localization of LPG from mutant parasites as compared to V121. Expression of gp63, a surface molecule also implicated in attachment to macrophages, was unaltered. All 3 mutants caused disease when injected into genetically susceptible BALB/c mice but lesions developed at a much slower rate than those caused by the virulent V121 clone. This slow rate of lesion development did not correlate with promastigotes' ability to invade macrophages in vitro. Karyotype analysis showed that there was a reduction in the size of chromosome band number 2 in all 3 mutants. The differences in LPG and chromosome band 2 were retained by mutant clones following passage through mice, suggesting that these phenotypes are stable.

Although the mutant parasites were infective and caused lesions, the changed structure of the LPG appeared to influence the virulence of the parasites.

PMID: 8008453 [PubMed - indexed for MEDLINE]

28: J Biol Chem. 1993 Apr 5;268(10):6909-16. Related Articles, Links

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Ricin-resistant Madin-Darby canine kidney cells missort a major endogenous apical sialoglycoprotein.

Le Bivic A, Garcia M, Rodriguez-Boulan E.

Biologie de la Differenciation Cellulaire, Unite de Recherche associee 179, Faculte des Sciences de Luminy, Marseille, France.

gp114 is a major sialoglycoprotein expressed on the apical membrane of Madin-Darby canine kidney (MDCK) II cells. We investigated its distribution in two lectin-resistant mutant cell lines derived from MDCKII cells, MDCKII-RCAr and MDCKII-ConAr cells. gp114 was present on the apical membrane of MDCKII-ConAr cells but was predominantly basolateral in MDCKII-RCAr cells. No change of polarity was observed for several apical and basolateral markers in this cell line. Reversal of polarity of gp114 mainly resulted from a modification of its intracellular sorting. gp114 showed altered endocytosis in MDCKII-RCAr cells. In MDCKII cells gp114 was slowly endocytosed, whereas in MDCKII-RCAr cells endocytosis of gp114 was highly increased. Using mannosidase I and II inhibitors we found that N-glycosylation only slightly affects gp114 sorting and endocytosis. Our results suggest that gp114 or an associated component in MDCK-RCAr fails to express apical information or that a mutation creates a basolateral sorting signal which could be related to endocytic signals.

PMID: 8463221 [PubMed - indexed for MEDLINE]

29: J Biol Chem. 1992 Dec 25;267(36):25939-44. Related Articles, Links

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Functional integrins from normal and glycosylation-deficient baby hamster kidney cells. Terminal processing of asparagine-linked oligosaccharides is not correlated with fibronectin-binding activity.

Koyama T, Hughes RC.

National Institute for Medical Research, Mill Hill, London, United Kingdom.

We have examined the properties of the alpha 5 beta 1 integrin of baby hamster kidney (BHK) cells, a ricin-resistant variant Ric14 lacking N-acetylglucosaminyl transferase I, and hence unable to complete assembly of hybrid- or complex-type N-glycans, and BHK cells treated with 1-deoxymannojirimycin (dMM), an inhibitor of Golgi mannosidases involved in the initial processing of N-glycan precursors. Comparable amounts of alpha 5 beta 1 integrin were isolated from these cells by chromatography of detergent extracts on a fibronectin cell-binding fragment affinity column and elution with EDTA. The alpha 5 beta 1 integrin obtained from normal BHK cells by fibronectin affinity chromatography contained mainly endoglycosidase H-resistant oligosaccharides, whereas in RicR14 cells or dMM-treated BHK cells these were entirely endoglycosidase H-sensitive. Analysis of lactoperoxidase labeled or long term biosynthetically 35S-labeled proteins from cultures of normal or glycosylation deficient cells showed similar steady state levels of alpha 5 beta 1 integrin and expression at the cell surface. Pulse-chase experiments in normal BHK cells showed rapid conversion of the alpha 5 subunit into a mature form containing oligosaccharides resistant to endoglycosidase H and slower maturation of a precursor beta 1 subunit, as in other cell types. In Ric14 cells the precursor beta 1 subunit was found to carry glycans larger than the fully processed Man5GlcNAc2 glycan of the mature subunit, indicating that the bulk precursor pool had not been translocated into the cis-Golgi compartment containing mannosidase I. We conclude that in BHK cells terminal oligosaccharide processing of alpha 5 beta 1 integrin subunits is not required for dimer formation, surface expression, and fibronectin binding, and that expression of the glycosylation defect of Ric14 cells on the alpha 5 beta 1 integrin does not account for the reduced adhesiveness of these cells on fibronectin compared with normal and dMM-treated BHK cells.

PMID: 1464606 [PubMed - indexed for MEDLINE]

30: J Immunol. 1981 Nov;127(5):2095-101. Related Articles, Links

Immunoglobulin glycopeptides from an IgG2b-producing mouse myeloma cell line and from variant cell lines.

Weitzman S, Portmore J.

A mouse myeloma cell line, 45.6.3, produces an IgG2b immunoglobulin (Ig) with 2 carbohydrate attachment sites on the heavy chains. One site is in the CH2 domain and the other in the VH region. The oligosaccharides at each site have different structures. The ratio of radioactive glucosamine incorporated in the VH compared with the CH2 oligosaccharide is approximately 1 to 3. In an attempt to understand this observation further, variant cell lines derived from 45.6.3 were

isolated and their Ig were characterized. A ricin-resistant line, R4R1.5, has the same 2 attachment sites as the wild type, but the ratio of radioactive glucosamine in VH compared with CH2 was 1:1 and not 1:3 as in the wild type. This alteration is most probably due to cellular factors, since the Ig protein is unchanged. The M3.11 cell line produces an Ig with a polypeptide deletion involving the CH3 domain. In this Ig, a 3rd carbohydrate attachment site can be demonstrated. The percentage of radioactivity glucosamine in the CH2 domain compared with the total Ig is about 25% instead of 75% as in the wild type. These results suggest that the extent of glycosylation of different sites on Ig can be affected by both cellular factors and structural changes in the Ig protein.

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31: Eur J Biochem. 1981 Jul;117(2):275-84. Related Articles, Links

Glycosyl transferases of baby-hamster-kidney (BHK) cells and ricin-resistant mutants. N-glycan biosynthesis.

Vischer P, Hughes RC.

Five cell lines of ricin-resistant BHK cells have been assayed for gross carbohydrate analysis of cellular glycoproteins, for the activities of several glycosidases and of specific glycosyl transferases active in assembly of N-glycans of glycoproteins. The latter enzymes include sialyl transferase using asialofetuin as glycosyl acceptor, fucosyl transferases using asialofetuin and asialoagalactofetuin acceptors, galactosyl transferases using ovalbumin, ovomucoid and N-acetylglucosamine as acceptors and N-acetylglucosaminyl transferases using ovalbumin and glycopeptides as acceptors. Cell line RicR14, binding less ricin than normal BHK cells, contains reduced amounts of sialic acid, galactose and N-acetylglucosamine in cellular glycoproteins and lacks almost completely N-acetylglucosamine transferase I, an essential enzyme in assembly of ricin-binding carbohydrate sequences of N-glycans. These cells also contain reduced levels of N-acetylglucosamine transferase II active on a product of N-acetylglucosamine transferase I action. Sialyl transferase activity is severely depressed while fucose-(alpha 1 leads to 6)-N-acetylglucosamine fucosyl transferase activity is increased. Cell lines RicR15, 17, 19 and 21 showed partial deficiencies in galactosyl and N-acetylglucosaminyl transferases. A hypothesis is put forward to account for the different carbohydrate compositions and ricin binding properties of glycoproteins synthesised by these cells in terms of the determined enzyme defects, the normal level of sialyl transferases detected in RicR15 and RicR21 cells and the elevated levels of sialyl and fucosyl transferases detected in RicR17 and 19 cells. None of the above changes in glycosyl transfer reactions in the RicR cell lines are due to enhanced glycosidase or sugar nucleotidase activities in the mutant cells.

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